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Wood and phloem formation in beech (*Fagus sylvatica*)

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Introduction

The increasing need to better understand the physiological processes of trees in response to environmental conditions, and the consequences of these conditions on wood quality, require comprehensive wood-formation studies in different environments all over the world (Deslauriers et al. 2003, Rossi et al. 2003, Gričar and Čufar 2008) not only on cellular but also on sub-cellular and ultrastructural level. These studies can build a link between different disciplines dealing with the environment, forests, trees and wood (Fonti and Jansen 2012).

Wood with its various cell types is produced by the cambium, and its quality is to a great degree defined during its formation and subsequent developmental processes. In addition, cambium produces phloem which translocates products of photosynthesis and is consequently crucial for tree survival. Nevertheless, phloem formation has so far been less investigated, partly also because of its lower economic importance.

Studies on beech (*Fagus sylvatica*) have shown relations between cambial activity, tree-ring width/anatomy variation and leaf phenology (Čufar et al. 2008b, Čufar et al. 2012). These processes are affected by climatic change which is especially pronounced in Slovenia, a transitional area between the Mediterranean, Alpine and continental climatic regimes (de Luis et al. 2012).

The aim of our contribution is to overview the main results regarding xylem and phloem formation in beech (*Fagus sylvatica*) from sites in Slovenia, with an emphasis on observations at different levels, e.g. cellular, sub-cellular and ultrastructural one.

Materials and methods

Investigations in beech (*Fagus sylvatica* L.) were performed on mature forest trees from two areas with temperate and Alpine climatic regimes in Slovenia: Panška reka near Ljubljana (400 m a.s.l.) and Menina planina in the Alps (1200 m a.s.l.) (e.g. Prislan et al. 2013b). The low-elevation site is representative of a large part of beech sites in Slovenia (Čufar et al. 2008a). The high-elevation site is located near the altitudinal limit of beech distribution in the Slovenian Alps. Investigations were conducted between 2006 and 2011. Similar studies still continue.

At both sites leaf phenology was observed as well. The dates of leaf unfolding and autumnal leaf colouring were recorded for the selected trees. The observations were made in accordance with Guidelines for plant phenological observations (Koch et al., 2007). All samples (inner bark, cambium, and outer xylem) were collected at weekly intervals from stems of living trees (at breast high) by the intact tissue sampling method (Gričar et al. 2007) or by micro-coring (Rossi et al. 2006). Immediately after removal from the trees, the samples were fixed in a solution of formalin, ethanol and acetic acid (FAA) and embedded in paraffin. Approximately 12 µm thick transverse sections were prepared using a rotary microtome, and stained with safranin and astra blue. The sections were examined with a light microscope (LM) (Fig. 1a, b, c). Different stages of xylem growth ring formation / cell differentiation were determined using bright field and polarised light (e.g. the phase of postcambial growth and the phase of secondary cell wall synthesis could be

most clearly seen under polarized light). The number of cell layers in the cambium and the widths of xylem and phloem growth rings were measured with an image analysis system.

Detailed ultrastructural and topochemical analyses of xylem, phloem and cambium were performed with a UV – microspectrophotometer (UMSP) (Fig. 2) and a transmission electron microscope (TEM) on samples embedded in epoxy resin. Depositions of cell-wall layers in xylem and phloem cell walls were observed with TEM on ultra-thin (90 – 100 nm) sections stained with potassium permanganate (Fig. 1e). In addition, lignin contents in xylem and phloem cell walls were determined semi-quantitatively on semi-thin sections (1 μm) with UMSP. For observation of seasonal ultrastructural changes in cambial cells, samples were prefixed with a glutaraldehyde/paraformaldehyde solution, postfixed with osmium tetroxide, and embedded in epoxy resin. Sections were then stained with uranyl acetate and lead citrate (Frankenstein et al. 2005) and examined by TEM (Fig. 1d).

Results and discussion

The results show that at the low-elevation site the production of new xylem cells in cambium in the period between 2006 and 2011 always started in the first half of April. The maximum rate of xylem-cell production occurred at the beginning of June. Cessation of xylem production was observed in mid-August. The patterns of wood-formation processes were similar at both locations, but there were differences in their timing. At the high-elevation site production of new cells started on average 1 month later than at the low elevation site, i.e. in mid-May, and maximum cell production occurred around the summer solstice. Xylem production at high elevation ceased slightly earlier than at the low elevation, i.e. in the first part of August. As a consequence, xylem increments were on average two times higher, and variation between different years was larger in trees at the low-elevation site in comparison to high elevation site (Čufar et al. 2008b, Prislan et al. 2013b).

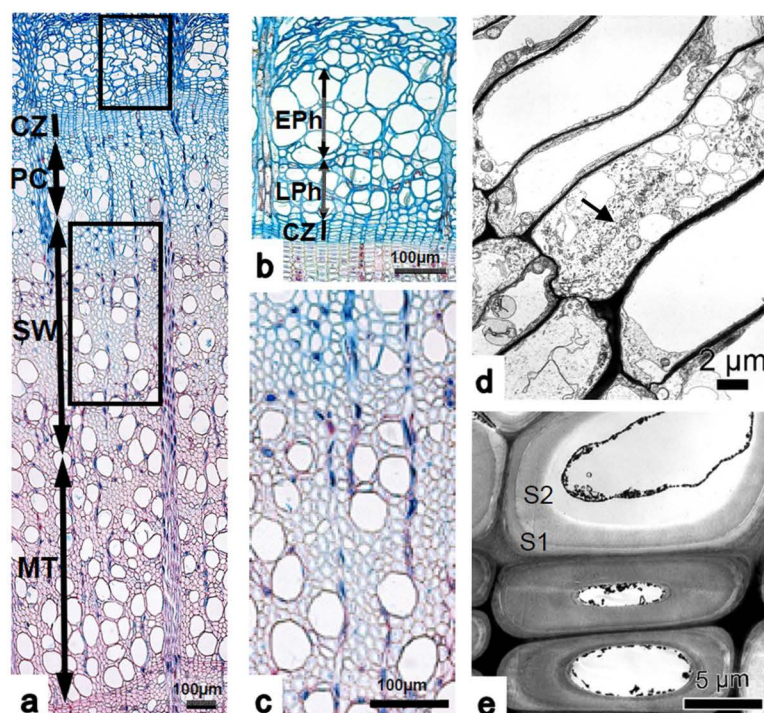


Figure 1: Xylem, cambium and phloem of *Fagus sylvatica* in different phases of development: (a) cross-section of the cambium (CZ) and developing xylem with cells with primary wall (PC), with developing secondary cell wall (SW), and mature cells (MT); (b) cambium and last formed non-collapsed phloem with early- (EPh) and late phloem (LPh); (c) SW cells –detail; (d) TEM image of active cambial cells, arrow points to new formed cell plate; (e) TEM image of developing fibre containing S1 and S2, below two mature fibres.

UMSP and TEM analysis of xylem cell wall formation were carried out in 2008 at the low elevation site Panška reka. These two approaches enabled detailed insights into the processes of cell-wall thickening and lignification of xylem cells (Fig. 1e, Fig. 2). Lignification generally started 1-2 weeks after the formation of the first new cells. The process of differentiation in the first formed xylem vessels was finished one month after the onset of cell production in the cambium. However, differentiation of the first formed xylem fibres lasted for approximately two months. After the cambial cell division was finished, between the beginning and mid August, the differentiation of the terminal fibres continued for another four weeks. Vessel walls were characterised by a larger amount of strongly absorbing guaiacyl lignin, whereas fibre walls contained more syringyl units. The content of guaiacyl lignin was also higher in the terminal part of the annual ring. Different lignification dynamics in initial and terminal parts of the growth ring can be linked with the different chemical structure of lignin in cell walls of vessels (with a higher amount of syringyl lignin in the terminal part) and fibres (with a higher amount of guaiacyl lignin) (Prislan et al. 2009).

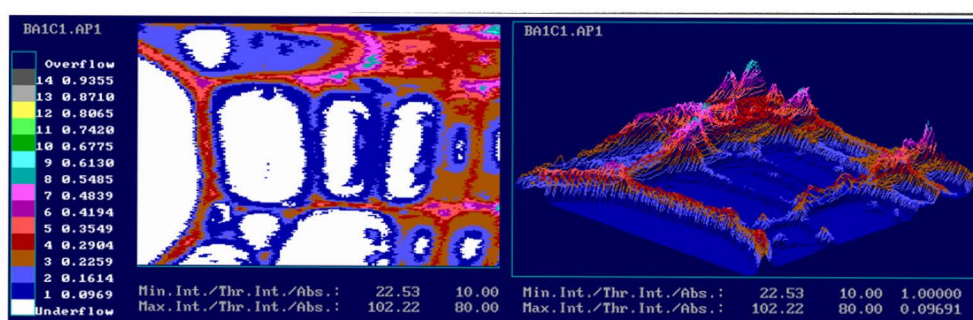


Figure 2: UMSP images of developing xylem fibres and vessels of *Fagus sylvatica*; the figure shows absorption values in fibre and vessel cell walls in the initial part of the growth ring in the middle of May (at the low elevation site). Different colours represent different absorption values; higher absorption values are related to higher lignin concentrations and vice versa. Since lignification starts first in cell corners (CC) and middle lamella (ML), the absorption values of these regions are higher. In S1 and S2 layers of the fibre cell walls the absorption values are low at that time because the lignification process is not finished yet, however in vessels the lignification process is almost finished. Legend – UV absorption at a wavelength of 280 nm and optical resolution of 0.25 μm x 0.25 μm .

Studies on the dynamics of phloem formation at low and high elevation sites revealed that the first new phloem cells were produced by the cambium at approximately the same time as the first xylem cells. However, phloem formation obviously started with the differentiation of 1-2 phloem cells which overwintered. The preparation techniques enabled us to produce microscopic slides where early phloem, late phloem and the phloem-ring boundary could be distinguished in the youngest non-collapsed phloem growth ring (Fig. 1b). It was shown that the collapse of early phloem sieve tubes occurs soon after the cessation of the cambium in August, indicating that at the breast height where the samples were taken they remain functional only during the current growing season (Prislan et al. 2012). The analysis of bark tissues showed that the proportion of sclereids increased with the age of the phloem. The chemical composition of lignin in sclereid walls proved to be similar to lignin in xylem-fibre walls; however, lignin concentration was higher in sclereids (Prislan et al. 2012).

The active cambium always contained more cells per radial row than the dormant one. Observations with TEM, revealed differences in the ultrastructure of cambial cells (Fig. 1d). We were able to differentiate between the phases of dormancy, reactivation, activity and transition to dormancy. Active cambial cells contained large central vacuoles, whereas the other cell organelles aggregated in narrow cytoplasmic strands attached to the cell wall. Furthermore, the cells contained thin walls, active dictyosomes with visible secretory vesicles, numerous spherical or oval-shaped mitochondria, mainly rough endoplasmatic reticulum of cisternal form, and numerous

plastids containing starch. Often newly forming cell walls could be seen in the middle of the dividing cambial cells (Fig. 1d). Cells in the dormant cambium were characterized by slightly thickened radial and tangential cell walls and contained lipid droplets. Plastids containing starch were rare, cytoplasm became denser, and the vacuoles were smaller and more numerous. According to these ultrastructural changes, cambial activity at both, the low and the high elevation site, started approximately one month earlier than determined by light microscopy (Prislan et al. 2011, Prislan et al. 2013a).

We showed that the onset of xylem and phloem cell production by the cambium coincided with leaf unfolding. According to long-term data (1955-2007), collected by the Environmental Agency of the Republic of Slovenia (ARSO), leaf unfolding in *Fagus sylvatica* is positively correlated to March temperatures at low elevations and with April temperatures at high elevations. As a consequence of climatic warming, significant trends towards earlier leaf unfolding were observed especially at high elevations (Čufar et al., 2012).

Comparison of results obtained by wood and phloem formation and leaf phenology studies can improve our understanding of the impact of environmental factors on radial growth in *Fagus sylvatica*.

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